

In the Specification

Please amend the specification as follows. No new matter is added by way of the changes.

Page 11, the first full paragraph:

The salt is present in the hypertonic, high-salt reagent at a high enough concentration effective to precipitate proteins out of a sample such that the proteins do not interfere in any subsequent downstream analysis of DNA. Preferably, the concentration of such salts is greater than about 1.0 M. More preferably, the concentration of such salts is greater than about 2.0 M. A preferred hypotonic, high-salt reagent is Puregene® PUREGENE® Protein Precipitation Solution (Gentra Systems, Inc., Minneapolis, MN, Cat. # D-5003, (Sodium Chloride > 5 M)). The hypertonic, high-salt reagent also serves to resuspend cells into solution, thus making the cells available for more efficient subsequent lysis. For example, addition of the hypertonic high-salt reagent to a cell pellet causes the cells to be resuspended almost instantaneously without the formation of aggregates or clumps. Furthermore, the use of the hypertonic, high salt reagent obviates the need for a pre-wash solution, such as a hypertonic wash solution to remove lipids and other contaminants from solution.

Page 12, the first full paragraph:

In one embodiment of the invention, the method includes the lysis of biological material such as cells or viruses which is achieved by combining biological material, comprising cells (or viruses), with a lysing reagent containing an anionic detergent to form a lysate. The lysing reagent is added after the aforementioned hypertonic, high-salt reagent is added to the biological material. As used herein, "lysis" refers to the destruction of a cell by rupture of its membranes. A "lysis reagent," generally includes, but is not limited to, an anionic detergent dissolved in a buffer. The reagent is buffered up to a pH of less than about 10, and preferably, less than about 9. Preferably, the pH of the lysis reagent is maintained at less than about 9 using a

buffer, such as Tris-[hydroxymethyl] aminomethane-ehtylenediamine acetic acid (Tris) buffer, although a Tris buffer is not a requirement as long as the buffer is capable of providing a pH of less than about 9 in aqueous media. However, any suitable buffer, known to those skilled in the art may be used. Suitable anionic detergents are those that are soluble in water at a level of at least about 0.1% weight/volume, based on the total volume of the reagent, and are capable of lysing cells and/or solubilizing proteins and lipids at this concentration. Such anionic detergents include, but are not limited to, salts (*e.g.*, sodium, potassium, and lithium salts) of dedecyl sulfate. Preferably, the anionic detergent is a dodecyl sulfate salt. Preferably, the concentration of such a dodecyl sulfate salt is greater than 0.1% w/v. This reagent lyses cells and viruses to form a lysate. A preferred lysis reagent is Puregene® PUREGENE® Cell Lysis Solution (Gentra Systems, Inc., Minneapolis, MN, Cat. # D-5002; 0.5% SDS, 0.1 M Tris, 0.1 M EDTA).

Page 13, the second full paragraph:

The first ancillary reagent is a red blood cell lysing reagent ("RBC Lysing Reagent") used when the biological material comprises mammalian whole blood. The red blood cell lysing reagent is used to lyse red blood cells and facilitate subsequent isolation of DNA from the white blood cells contained in mammalian whole blood. This reagent is referred to herein as the "RBC lysis reagent" and comprises compounds such as ammonium chloride, sodium bicarbonate, and EDTA in deionized water at concentrations sufficient to lyse red blood cells preferentially over white blood cells. Such concentrations are known to those skilled in the art. In particular, the RBC lysing reagent causes the preferential rupture of the cellular membrane of red blood cells, while having no significant lysing effect on the cellular or nuclear membranes of white blood cells. The RBC lysing reagent is added to mammalian whole blood which causes the red blood cells to lyse, leaving behind substantially intact white blood cells in solution. The white blood cells (and any cell-associated viruses that may be present) are then separated from the red blood cell lysate by centrifugation or

any other appropriate method and the red blood cell lysate discarded, after which the hypertonic, high salt reagent is directly added to the white blood cells. Subsequently, a lysis reagent is added to rupture the membrane thus releasing the DNA. A preferred lysis reagent is the Puregene® PUREGENE® RBC Lysis Solution (Gentra Systems, Inc., Minneapolis, MN, Cat. # D-5001; 145 mM ammonium chloride, 0.5-2 mM sodium bicarbonate, and 0.75-1.25 mM EDTA). In the current invention, the RBC Lysing reagent is only used to lyse red blood cells. It is not used to resuspend white blood cells or any other biological material before cell lysis.

Page 14, the first full paragraph:

Other ancillary reagents can include but are not limited to alcohols, *e.g.*, 100% isopropanol to precipitate DNA, 70% ethanol to wash the precipitated DNA, RNases such as RNase A to remove RNA, Proteases such as Proteinase K to digest proteins, glycogen to facilitate DNA precipitation and recovery, and a DNA hydration reagent. A DNA hydration reagent is used to dissolve the isolated and purified DNA for storage. If stored in the DNA hydration reagent, the isolated DNA can be stored for an indefinite period of time at temperatures of -20 or -80 °C. Preferably, a DNA hydration reagent comprises a buffer, *e.g.*, a buffer with 10 mM Tris, and 1 mM EDTA, pH of about 7- - 8. A preferred DNA hydration reagent is Puregene® PUREGENE® DNA Hydration Solution (Gentra Systems, Inc., Minneapolis, MN, Cat. # D-5004; 10 mM Tris, 1 mM EDTA pH 7.0-8.0).

Page 17, the third full paragraph continued on Page 18:

A blood sample collected in a standard blood collection bag was obtained from the Memorial Blood Centers of Minnesota and stored at 4°C until use. DNA was purified from the blood samples within 96 hours of being drawn. DNA was purified using the Puregene® PUREGENE® DNA Purification Kit (Cat No. D-50K, Gentra Systems, Inc., Minneapolis, MN) using both the standard 10 ml whole blood protocol and the

new rapid purification protocol. This kit contained the following Puregene[®] PUREGENE[®] reagents: RBC Lysing Solution, Cell Lysis Solution, Protein Purification Solution, RNase A Solution, and DNA Hydration Solution. Additional reagents were also required for the procedure: 100% isopropanol (2-propanol) and 70% ethanol. Note that although the biological material described in this example is a white cell pellet from whole blood, this method is applicable to collected or pelleted cultured cells, virus particles, pellets from solid or liquid body fluids in a saline or detergent solution.

Page 20, the first paragraph continuing from Page 19:

A volume of 10 ml whole human blood was added to 30 ml RBC Lysis Solution (Gentra Systems, Inc., Minneapolis MN) in a 50 ml centrifuge tube. Each sample was inverted to mix and incubated 5 minutes at room temperature with one additional inversion half way through the incubation period. To collect the white blood cells, which contain genomic DNA, each sample was centrifuged for 2 minutes at 2,000 x g. The supernatant fraction was removed by decanting, leaving behind a visible white cell pellet and about 200 µl of the residual liquid should remain. White blood cell pellets are generally very difficult to resuspend in solution. Often vigorous stirring, mechanical agitation or resuspension in a PBS buffer or equivalent fails to resuspend the cells adequately, and the cells continue to clump or aggregate. Thus, to resuspend the white cells, 3.33 ml of a hypertonic, high-salt reagent was used (Puregene[®] PUREGENE[®] Protein Precipitation Solution (Gentra Systems, Inc., Minneapolis, MN, Cat. # D-5003, (Sodium Chloride > 5 M)) was dispensed vigorously into the center of the white cell pellets to disperse the white blood cell pellet. Unexpectedly, this forced the white blood cells to form a suspension immediately without any significant damage to the cells (as seen when analyzed under a microscope), and without any further physical dispersion steps. Immediately following this step, a volume of 10 ml cell lysis solution (Gentra Systems, Inc., Minneapolis, MN) containing RNase A Solution was added to the sample to lyse the

cells. RNase A solution is not required, but was used to provide a more direct comparison with the standard method. To prepare the combined cell lysis solution and RNase A solution, 5 ml of RNase A solution was added to 1000 ml of Cell Lysis Solution and mixed thoroughly prior to starting the procedure; this solution is stable for at least 6 months at room temperature. To mix the reagents with the resuspended cells and to complete cell lysis, each tube was vortexed vigorously for 20 seconds. Then each sample was centrifuged at 2000 x g for 2 minutes to collect the precipitated and other contaminants, which formed a tight dark brown pellet.